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Attorney Docket No. 24024-501CON

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

APPLICANTS:	Peled et al.		
SERIAL NUMBER:	09/463,320	EXAMINER:	Michail A. Belyavskyi, Ph.D.
FILING DATE:	January 22, 2000	ART UNIT:	1644
FOR:	METHODS OF CONTROLLING PROLIFERATION AND DIFFERENTIATION OF STEM AND PROGENITOR CELLS		

MAIL STOP RCE
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION OF DR. EITAN FIBACH UNDER 37 CFR 1.132

1. I am presently employed as researcher at the Hadassah University Hospital, Department of Hematology, where I am a full professor. I received my Ph. D. degree from the Weizmann Institute of Science in Rehovot, worked as a post-doctoral fellow at the Cancer Research Center, College of Physicians and Surgeons of Columbia University, New York, NY, USA, and was a visiting scientist at the Laboratory of Chemical Biology, NIDDK, National Institutes of Health, Bethesda, MD, USA. My Curriculum Vitae is attached.

2. My research focuses on the development of hematopoietic cells. Since the beginning of my career, I have published more than 150 scientific articles in highly regarded journals and books, and have presented my achievements at many international scientific conferences. I am a member of American Society of Hematology and The International Society of Experimental Hematology. I have served on the editorial board of the journal "Experimental Hematology".

3. I am a co-inventor of the subject matter claimed in the above-referenced U.S. patent application. I have read the Office actions issued with respect to the above-identified application.

4. In the most recent Office action, the Examiner has rejected claims 1-2, 4-13, 15,

37-45 and 47 under 35 U.S.C. § 112, first paragraph as containing subject matter which was not described in the specification in such a way as to enable one of ordinary skill in the art to make and/or use the invention. The Examiner states that while the specification clearly discloses methods of hematopoietic cells transplantation and adoptive immunotherapy by expanding and inhibiting differentiation of CD34+ cells ex vivo, the specification is not enabling for any hematopoietic cells. The Examiner further states that the specification does not teach how to effectively expand and at the same time inhibit differentiation of any hematopoietic cells, and that, the art being unpredictable, given the lack of guidance and working examples, it would take undue trial and error experimentation to practice the claimed invention.

5. I would like to emphasize that while expansion of hematopoietic cells is a well established technique in the art, the present invention is based on a novel method for expanding a population of undifferentiated hematopoietic cells for transplantation and at the same time inhibiting their differentiation, by addition of high affinity copper chelators to the culture medium, in both serum-free and serum-supplemented media, enabling more efficient expansion of stem/progenetitor cells as compared with methods previously disclosed in the art.

6. The Examiner has stated that the specification provides enablement only for CD34+ cells that were first isolated (enriched), and then used for ex vivo expansion with the methods of the present invention, using copper chelators and cytokines. I wish to take issue with the Examiner's assertion: in the studies and experiments using the protocols of the present invention and which are described in the specification, we demonstrated, for the first time, that the use of copper chelators is effective in enhancing expansion and inhibiting differentiation in both the CD34+ and murine erythroleukemia undifferentiated cell cultures examined. We demonstrated further that copper chelators have a significant effect on differentiation and growth inhibition of all of the undifferentiated and differentiated cell cultures examined (murine erythroleukemia cells, erythroid precursors form peripheral blood, mononucleocytes, hepatocytes, and embryonal stem cells). Thus, I believe that the specification discloses methods for ex-vivo expansion of and inhibition of differentiation of stem/progenitor cells from a broad range of cells.

7. In another set of experiments described in the specification, I observed that, following our protocols for hematopoietic reconstitution of lethally irradiated mice with bone marrow cells expanded ex vivo with the copper chelator TEPA and cytokines, the mice reconstituted with chelator-expanded bone marrow cells achieved superior WBC recovery and survival as compared to cells expanded with cytokines alone.

8. The Examiner has stated that the instant specification does not teach how to extrapolate data obtained from CD34+ cells ex-vivo assay studies to the development of effective protocols for imposing proliferation and at the same time restricting differentiation of any stem and/or progenitor cells. I would like to present the results of recent experiments using the protocols of the present invention for expansion and inhibition of another undifferentiated cell population, AC 133+ cells.

9. AC 133+ cell lines are important cells in the field of hematopoietic stem cell differentiation. The recent identification and isolation of human hematopoietic cells expressing AC133, has provided a better understanding of the relationships between the cell surface phenotype of the subpopulations comprising the human hematopoietic system and their proliferative and differentiative capacity (see, for example, Bhatia, M., Leukemia 2001; 15:1685-88). Studies of cultures of the AC 133+ subpopulation indicate that AC133+ cells have high self-renewal capability, maintain early hematopoietic stem/progenitor cell (HSPC) characteristics, and show superior survival in culture, as compared to CD34+ cells (see Forraz, et al, Br. J. Haematology, 2002;119:516-24). Stem cell selection, and many transplantation protocols have now been amended to include identification of candidate cells as AC 133+ as well as CD34+. In a recent series of experiments, we have found that the methods of the present invention for expansion and inhibition of differentiation of cells for transplantation could be easily applied to AC133+.

10. In the experiments described in Appendix I, we compared the results of the application of the methods of the present invention for expanding hematopoietic cells selected according to CD34+, to those selected according to AC 133+. Cord blood derived AC 133+ or CD34+ cells were expanded in culture medium containing MEMα and 10% FCS, thrombopoietin (TPO), interleukin-6 (IL-6), FLT-3 ligand and stem cell factor (SCF),

each at a final concentration of 50 ng/ml, and 5 μM tetraethylenepentamine (TEPA) (Appendix I, Table 1).

11. As a first step towards large scale experiments and a clinical trial we replaced a research grade **CD34**-based separation device with a clinical grade CliniMACS in combination with the **AC133** CliniMACS reagent. For this purpose, we compared the cell yields and purity following progenitor cell purification utilizing both **CD34** and **AC133** reagents.

12. Cord Blood Units (CBUs) were thawed and the cells were divided into two equal fractions. The enrichment efficacy in terms of cell yield (number of eluted cells) and purity (percent of **CD34+** or **AC133+** cells) was comparable following enrichment on CliniMACS utilizing either **CD34** or **AC133** reagents (Appendix I, Fig. 1). Indeed, more than 90% of the **AC133+** cells were also positive for **CD34**. A representative FACS analysis is shown in Appendix I, Fig 2. CFUc frequency was also comparable in the progenitor cell fraction enriched by the **AC133** reagent to the fraction enriched by the **CD34+** reagent: 0.2±1.1 and 0.12±0.7, respectively.

13. We then compared 3 week parameters of cultures (n=4) initiated with a total of 2.5 x10^5 **AC133+** or **CD34+** cells derived from the same CBU. The yield of TNC was 1065 x 10^5 ± 124 and 760 x 10^5 ± 75, CFUc 81 x 10^5 ± 9 and 83 x 10^5 ±12, **CD34+** cells 43±7 x 10^5 and 39±9 x 10^5, **CD34+**CD38- cells 12±3.6 x 10^5 and 5.6±1.3 x 10^5, respectively (Appendix I, Fig. 3). In addition, similar proportions of cells expressing myeloid, lymphoid and megakaryocytic phenotype were found in cultures initiated either with **CD34+** or **AC133+** cells (Appendix I, Fig 4).

14. Based on these experiments we used the **AC133** CliniMACS reagent in combination with the CliniMACS separation device for the large-scale evaluation of our three weeks expansion procedure.

15. The **AC133** antigen is an important marker for stem/progenitor cells. Numerous phenotypic and functional studies demonstrate that the **AC133**-enriched population could serve as an alternative approach to **CD34+** cell selection and engraftment purposes.

Furthermore, AC133+ selected cells have been already used in clinical transplantation settings without any safety impairment. Accordingly, our first line of experiments demonstrated similar performance using the CliniMACS instrument and the "MiniMACS CD34 progenitor cell isolation kit" (data not shown).

16. For successful ex vivo expansion, both the number of cells recovered following the enrichment procedure and the purity of the progenitor cell fraction are two crucial parameters. Our results demonstrated similar yield and purity of the fractions recovered following the CliniMACS enrichment procedure utilizing either CD34 or AC133 reagent. Furthermore, following a three week large-scale clinical grade expansion, the yield of CB-derived early progenitor cells in culture initiated with $2.5x10^5$ AC133+ cells was statistically similar with that initiated with a same number of CD34+ cells.

17. Thus, not only CD34+, but also AC 133+ cells cultured in the presence of copper chelator, according to the methods of the present invention, exhibit efficient expansion and inhibition of differentiation of hematopoietic stem/progenitor cells. Along with the Examples described in the specification, I believe these results clearly indicate that the methods of the present invention can be successfully extrapolated to populations of undifferentiated cells other than CD34+ cells.

18. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United states Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

E. Fibach

Prof. Eitan Fibach

Signed this day 26 of November, 2003

Enc.:
Curriculum Vitae of Prof. Eitan Fibach
Appendix I

APPENDIX 1

Fig 1:

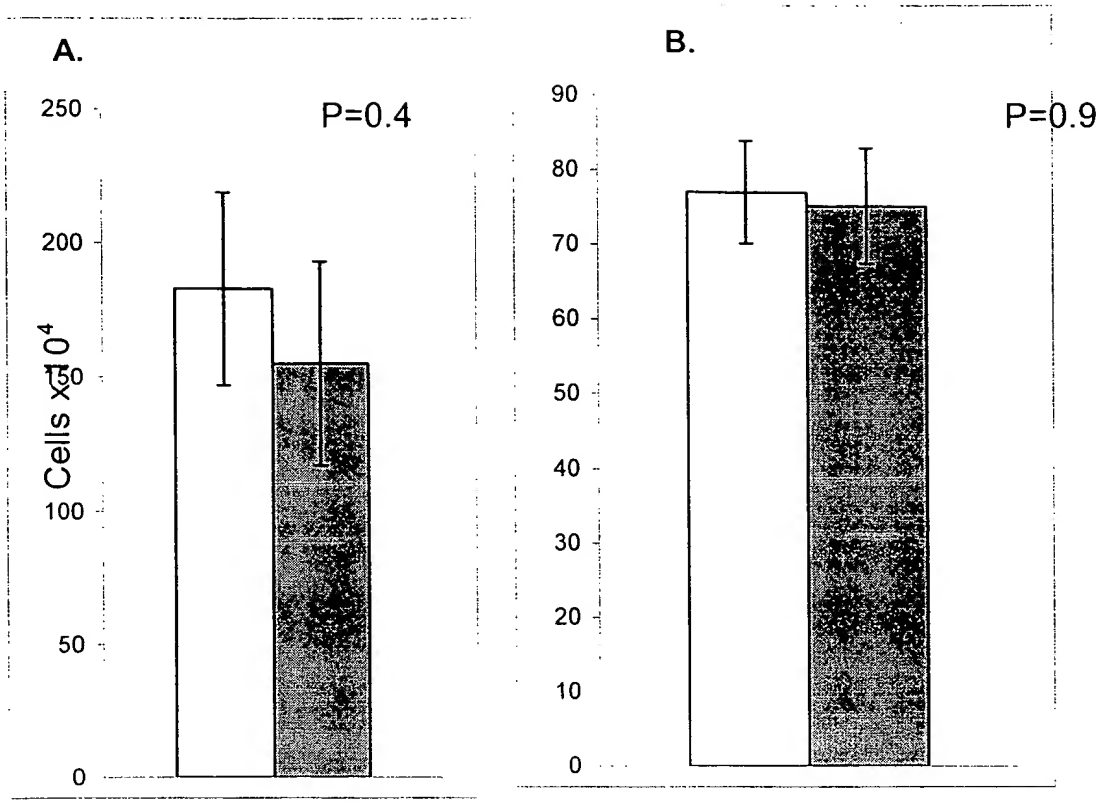


Fig 2:

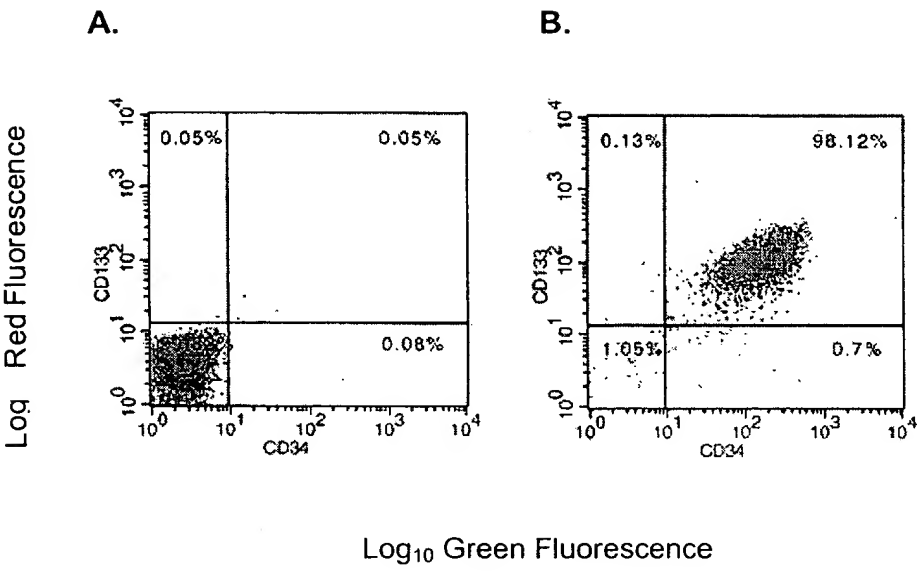

A.
B.
0.05%
0.05%
0.08%
0.13%
98.12%
1.05%
0.7%
CD133
CD34
Log Red Fluorescence
Log10 Green Fluorescence

Fig. 3a-e: Ex vivo performance of 3 week cultures initiated with either CD34+ or AC133+ enriched cells n=4

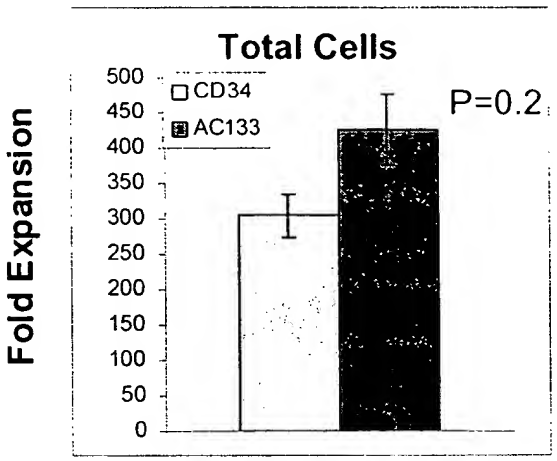


Fig. 3a

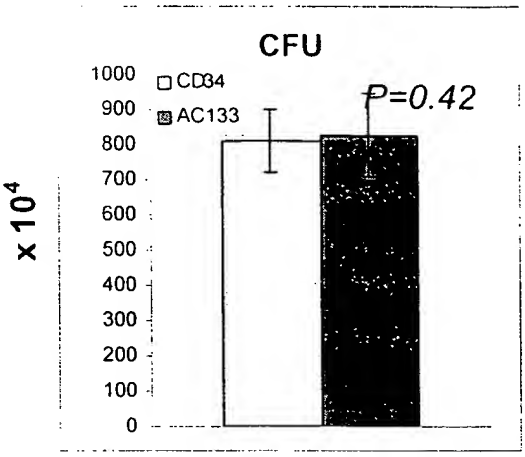


Fig. 3b

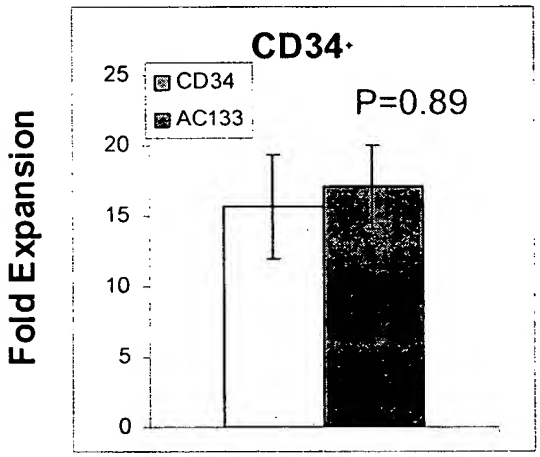


Fig. 3c

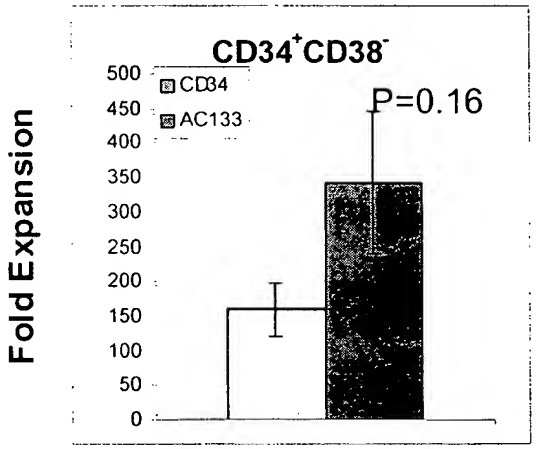


Fig. 3d

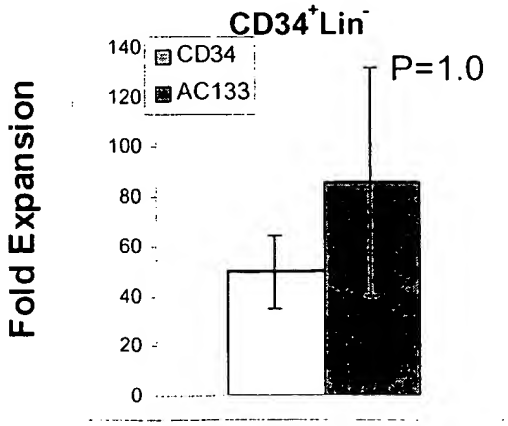
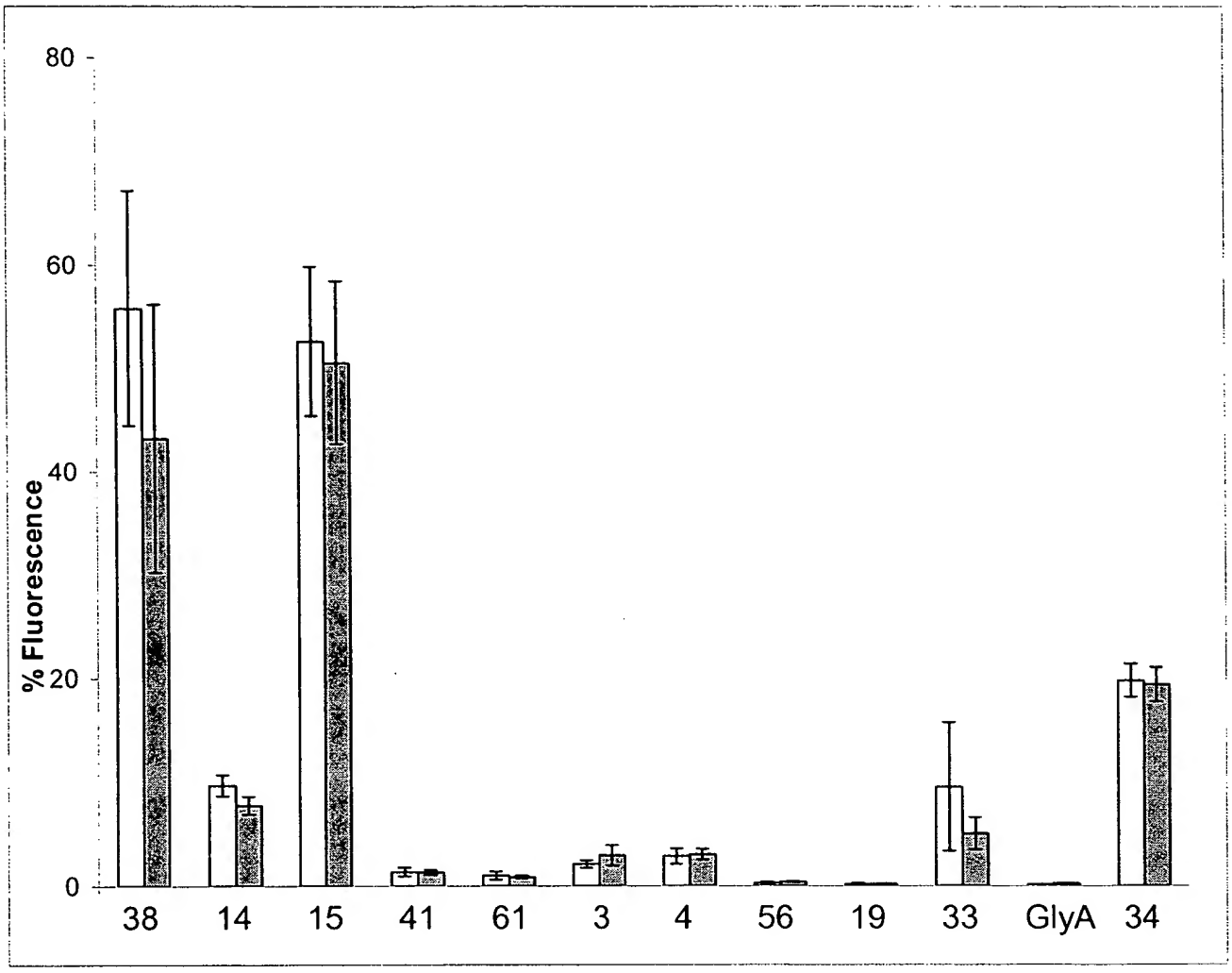


Fig. 3e

Fig 4:



CURRICULUM VITAE **Eitan Fibach, Ph.D.** September, 1999

Ap. A

Date/ place of birth:	June 25th, 1944, Tel-Aviv, Israel.
Nationality:	Israeli.
Marital Status:	Married plus 2 children.
1962-1965	Military Service.
1965-1970	M.Sc. studies, Department of Microbiology, Tel-Aviv University, Israel.
1968-1969	Consultant in Industrial Mierobiology, Decco Food Company, Kibbutz Bror-Hail, Israel.
1969-1970	Research, Central Virology Lab., Ministry of Health, Tel Aviv, Israel.
1970-1975	Ph.D. studies and research with Prof. Leo Sachs, Department of Genetics, Weizman Institute of Science, Rehovot, Isreal. Subject of research: Induction of differentiation in myeloid leukemic cells.
1975-1978	Post-doctorate research with Dr. Paul Marks, Cancer Research Center and Department of Human Genetics and Development, College of Physicians and Surgeons of Columbia University, New York, NY, USA. Subject: Regulation of differentiation in transformed erythroid cells.
1978-	Senior Investigator, Dept. of Hematology, Hadassah University Hospital, Jerusalem, Israel. In charge of the Hemopoiesis and Flow cytometry units.
1982-	Department of Biology, The Open University, Tel Aviv, Israel.
1984-1985	Scientific Consultant - Biotechnology General Inc., Rehovot, Israel.
1984-1987	Senior Lecturer, The Hebrew University - Hadassah School of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.
1987-1996	Associate Professor, The Hebrew University - Hadassah School of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.
1990-1995	Visiting Scientist, Laboratory of Chemical Biology, NIDDK, National Institutes of Health, Bethesda, MD, USA.
1995-1996	Vice President for research, C. P. Li Biomedical Research Co., Arlington, VA, USA.
1996-	Full Professor, The Hebrew University - Hadassah School of Medicine, The Hebrew University of Jerusalem, Jerusalem, Israel.

Membership in International Societies:

- International Society of Experimental Hematology.
- American Society of Hematology.
- American Society for Photobiology.

Editorial Boards

- Experimental Hematology.
- Cancer Therapy and Control.

September 1999

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